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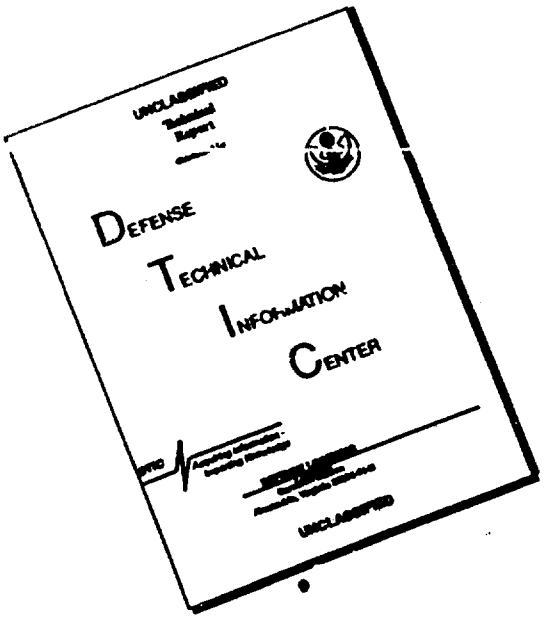

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Culturing Rabies (Hydrophobia) Viruses in a Developing Chicken Embryo, by M. A. Selimov and E. V. Semenova (Virus Div. Moscow Scientific Study Institute of Vaccines and Serums, I. I. Mechnikov, Moscow)

Soviet and foreign researchers many times have undertaken experiments in cultivating rabies virus in a developing chicken embryo (1-6).

Some of them were not able to prove that there was multiplication of the rabies virus (1) and studies of others (4, 5), even though they came to positive results, did not continue them. The exception is the works of Kogrowski and Cox (6) on cultivation of the strain Flury in an organism of a chicken embryo; this strain is being used successfully at the present time abroad for massive prophylactic vaccination of dogs. As a result of 136 intrabrain passages this virus strain was preliminarily adapted to the organism of chicks of one day age. Only after this was the strain Flury cultivated in a chicken embryo by injection into the yolk. After 40-50 passages through the organism of a chicken embryo the strain appeared fully apathogenic for dogs and rabbits during intramuscular introduction. A greater weakening of the pathogenicity of this strain was noted after 120 passages when it appeared apathogenic for grown mice during introduction into the brain. During this the strain Flury fully retained its immunogenic characteristics. It was established that immunity in dogs, caused by a single injection of the anti-rabies vaccine prepared from the strain Flury, is retained for 3 years while introduction of an inactivated neurotissue vaccine is retained no more than one year (6).

The egg anti-rabies vaccine has a very important meaning also in use as a specific prophylactic of rabies in man. This vaccine does not contain nerve tissues which evidently will have importance for prophylactics of postvaccinal

paralysis.

In this report are set forth data on the isolation and cultivation in the organism of a developing chicken embryo of a new strain of virus of street rabies.

The original strain was isolated from the brain of a dog which had died from rabies. White mice, infected in the brain and muscles with a 10% suspension of the brain of that dog, became ill with typical clinical appearances of rabies, and in cuts of their brain there were detected large quantities of Negri bodies. This strain of virus, named by us strain 83, was also identified by reaction of neutralization with immune anti-rabies serum. Index of neutralization of the original strain with a dilution of serum 1:200 was equal to 316, the egg strain after 16 passages - 1700. The fluctuation of the index of neutralization is explained by the diverse action of the anti-rabies serum.

For the infection of chicken embryo there was used a 10% suspension of brain of dogs which perished when stored at 15°C. The chicken embryo, incubated at 37°C for 7-8 days, with accurate maintenance of the asepsis, was injected in the brain (through the opening of the shell in the region of the air space) with 0.05 ml with the aid of a tuberculin syringe with a very thin needle. The embryo, incubated for 9-10 days at 36-38 C, was then opened in the usual manner. From the various tissues of the embryo there were prepared respective suspensions with distilled water and the presence of virus of rabies in them was determined by injection into the brain of white mice weighing 7-8 grams. A 20% suspension of the brain of the chicken embryo was used in further passages. There were conducted 19 successive passages on the brain and 6 on the yolk; that is, 25 in all.

Summary data on the cultivation of the strain 83 in chicken embryo are set forth in Table 1 Appendix. As Table 1 shows, during the course of 23 successive passages of the suspension of the brain and body of the chicken embryo, which

had been infected with strain 83, there was a continual appearance in the mice of a typical clinical chart of rabies. Titer of virus (ID50 according to Reed and Mench) in the brain tissue of the chicken embryo attained 10-2.0 to 10-5.6.

Regarding the adaptation of the virus to the organism of the chicken embryo there was observed a shortening of the incubation period of infection in the mice.

Morphological analysis for Negri bodies, cuts of the brain of the mice infected with the virus, in various degrees of adaptation to the organism of the chicken embryo, also indicate that the virus gradually becomes fixed in the organism of the chicken embryo. During analysis of paraffin sections of mice brains, tinted according to S. N. Muromtsev and E. I. Turevich, the Negri bodies were detected in large quantities only in those mice which had been infected with the original strain or with virus of the first passage. In preparation of the brain of mice infected with the egg strain the Negri bodies were detected with difficulty after the 8-16 passages. Their structure differed from the typical Negri body by the absence of internal structure, less so according to their possibility of being tinted and insignificant sizes.

It is necessary to note that the atypical Negri bodies were also found after the first passage in the brain of the chicken embryo. In preparations, strained according to S. N. Muromtsev and E. M. Turevich, there appeared single, fine, poorly tintable incorporations, distributed in the protoplasm of the nerve cells of the brain. Similar formations could be found in the brains of chicken embryos infected with virus to the 8th egg passage; after the 10th passage these incorporations were not detected in the brain of the chicken embryo.

Special tests were run to study the distribution of the rabies virus in the

various tissues of the chicken embryo.

The presence of virus in the tissues of the chicken embryo was determined by infecting mice weighing 7-8 grams with 0.03 ml into the brain. In all cases of the tests the embryo used was preliminarily incubated at 38°C for 7 days and injected in the brain with virus of the 8th or 10th passage and in the yolk of the 16th passage (at 0.25 ml). Preliminary results of the tests indicated that the rabies virus accumulated to a significant degree in the tissues of the brain and body of the chicken embryo (Table 2). A minimal quantity of virus was determined in the tissue of the allantois membrane and also in the amniotic and allantois fluid. There also was an insignificant finding of the rabies virus in the blood of the chicken embryo, which indicates virusemia.

In order to idnetify and also study the characteristics of the egg strain of rabies virus there were conducted tests on the infection of 14 non-pedigreed puppies (dogs) 1½ to 2 months old (Moscow Veterinary Division, A. V. Kovrigin and M. F. Kovalevski aiding).

For the infection with the egg strain of the rabies virus the region of the operation field, after shaving of the fur, was worked with a 10% tincture of iodine, then the skin and subskin cellular tissue was cut to the peritoneum in the usual manner. With the aid of an ordinary trephine, used for the infection of rabbits, a trephination was made of the skull in the region of the sinciput, 1.5 cm in the direction of the central line of the skull from the external corner of the eye-socket, and through the trephine aperature, under the hard brain membrane, was introduced 0.25 ml of a 20% suspension of brain of chicken embryo which had been infected with rabies virus. Infection of the pups in the masticatory muscle, in the muscle of the thigh and also under the skin in the region

of the internal surface of the groin were made in the regularly applied fashion.

According to Table 3, of 8 pups infected with egg strain of rabies virus of the 11-14th passages, 3 pups did not become infected with rabies. Of 4 pups infected with the egg strain of the 16th passage in the muscle and subcutaneous cellular tissue 1 pup did not get infected. These preliminary data of the weakening of the pathogenicity of the egg strain of the rabies virus demands verification on a large number of animals.

Pups infected with the original strain (No. 21, 22), and also with the egg strain, which was in various degrees of adaption to the organism of the chicken embryo (No. 11, 13, 14, 16, 18, 24, 25, 26), became infected with symptoms characteristic for the street rabies. The furious type of rabies was not observed in any cases, this can be explained by the age of the tested animals and also by the extra large dose used to infect them. Under the mixed form of rabies we understand those cases, the clinical of which is a clearly distinguished period of excitation and followed by a period of paralysis. The period of excitation lasted 1-2 days ordinarily. At first there was paralysis of the rear extremities or the lower jaw. In pup No. 13, along with the paralysis of the rear quarters, there was paralysis of the neck muscles. A clear paralytic form of rabies developed mainly in those pups which had been infected with the egg strain of the 16th passage in the muscle of the thigh and in the subcutaneous cellular tissue (pups No. 24, 25, 26). In the period of full paralysis there appeared in some of the pups approaches to convulsions which were caused by very light air movements or mechanical vibration (aerophobia).

Daily variations of the temperature in the rectum of the pups indicated that at the start of the infection the temperature increased regularly; 40°C, was the maximum temperature attained. In the period of paralysis the temperature fell

to normal or lower (Graph 1).

As can be seen on Table 3, the incubation period in pups infected with the egg strain proved to be shorter than the incubation period of the 2 pups infected with the original strain, which points to the partial fixation of the rabies virus strain which has been adapted to the organism of the chicken embryo.

More surprising data on the variability of the egg strain of the virus in an organism of a chicken embryo were noted during virusological analysis of the brain and submaxillary glands of the pups. According to Table 3, the titer of virus in tissues of the submaxillary glands of the pups infected with the original strain of virus of street rabies attained a dilution of 10-5.0 and the content of virus in tissue of the submaxillary gland of pup No. 21 was higher than the titer of virus in the tissue of the brain. At this same time, during experimental rabies caused by egg strain, no virus was detected in the submaxillary glands of the pups and, along with this, there was noted a high content of it in the tissue of the brain of the animals.

The data of the histological analysis are very interesting and also the analysis for the presence of Negri bodies in sections of the brain of the pups infected with the egg strain of the rabies virus. Portions of the brain of pup No. 14 and 18 were subjected to histological analysis. In the sections of these pups (hematoxylin-eosin method of stain) there were detected distributions of encephalitis and meningo-encephalitis of a nodule character; areas of proliferation of the macro- and microglia, perivascular infiltrates in the surface as well as in the deeper layers of the brain; in the ependyma of the brain ventricles, in the gray and white substance, in the Ammon's horn. Distinct variations of the nucleus were noted in the cells of the brain; shriveling, pyknosis and decentralization. In the substance of the brain there were encountered small sections of

hemorrhaging (studied by V. N. Kartashov).

Sections of the brain (Ammon's horn, cortex of the cerebellum and medulla oblongata) of all the ailing pups were subjected to morphological analysis for Negri bodies according to S. N. Muromtsev and E. N. Turevich. Data of Table 3 indicate that the sections of the brain of the pups infected with the original strain of the street virus of rabies contained large quantities of typical Negri bodies, but detection of the Negri bodies in these pups infected with the egg strain was far from regular.

The data of our tests fully agree with the material of Koprowski and Cox (6), but we differed from those researchers by the fact that we conducted satisfactory adaptation of the new strain of street virus of rabies to the organism of chicken embryo by injection into the brain. During this, in regard to adaptation of the virus to the organism of a chicken embryo, there was observed a variation (fixation) of the strain 83 which was reminiscent of the Flury strain, and namely; gradual shortening of the incubation period of infection in mice, weakening of the ability of the virus to bring about a formation of the Negri bodies in pups (dogs) and mice, disappearance of the virus from the tissue of the submaxillary gland of dogs, morphological variations of the nerve cells with a predominant infection of the nuclei and a paralytic form of rabies in a majority of the infected pups.

CONCLUSIONS

1. 19 successive passages of the rabies virus strain 83 through the brain of a chicken embryo and 6 passages through the yolk sac are feasible.

2. In the process of cultivation of the rabies virus in the organism of chicken embryo there was a variation-fixation of it; shortening of the incubation

period of infection, weakening of the ability of the virus to cause formation of the Negri bodies in the animals and disappearance of the virus from the tissue of the submaxillary gland of the pups (dogs).

Literature

1. Vinogradova, A. S. Journ. Microbiol., 1936, Vol. 17, No. 4, Pages 560-571.
2. Karnaukhova, E. L. In book: Transact. of the Sverdlovski and Permsk Inst. of Microbiol. and Epidem., Vol. 2, B. 1, Pages 117-123.
3. Pugach, E. P. and Nikitin, S. A. Jour. Microbiol., 1938, No. 4, Pages [illegible].
4. Bernkopf, N. and Kligler, I. J. Proc. Sc. Exper. Biol. a. Med., 1940, Vol. [illegible], Pages 332-335.
5. Dawson, J. R. Science, 1939, Vol. 89, Pages 300-301.
6. Koproswki, N. A. and Cox, H. R. J. Immunol., 1948, Vol. 60, No. 4, Pages [illegible]

Table 1 . Virulence of material of chicken embryo for mice during injection into the brain.

No. of passage	Suspension of brain of embryos: Morbidity	Titer of virus LD50	Days of incubation	Suspension of body of embryo: Morbidity	Titer of virus (LD 50)	Negri bodies
1	2/6	-	13	-	-	ppp
3	6/6	10-2.3	8	4/10	-	ppp
4	5/5	-	8	5/5	-	pp
5	4/9	-	9	-	-	pp
6	4/4	-	6	4/4	-	pp
7	6/6	10-5.3	6	5/5	10-2.3	pp
8	6/6	10-5.3	6	-	-	p
9	6/6	-	5	-	-	p
10	6/6	10-2.0	5	-	-	p
11	6/6	10-3.0	5	-	-	p
12	6/6	-	5	6/6	-	p
15	6/6	10-3.1	5	6/6	-	p
16	6/6	10-3.1	5	6/6	10-2.3	p
18	6/6	10-5.0	5	6/6	10-3.0	p
19	6/6	10-5.0	5	6/6	-	p
21	6/6	10-5.6	5	6/6	10-4.6	-
23	/66	10-4.9	5	6/6	10-4.6	0

Explanation: Numerator- Number of animals infected with rabies; Denominator- Number of animals taken in test; - not analysed; p very seldom detected atypical Negri bodies; p, pp, ppp- small, average and significant quantities of Negri bodies.

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Table 2 . Determination of rabies virus in an organism of a chicken embryo

Passages of virus	Titer (LD50) of virus in: Brain	Body	Amniotic membrane	Allantois membrane	Yolk membrane	Amniotic membrane	Allantois membrane	Blood
8th	10-3.0	10-2.3	10-2.0	-	-	10-0.9	-	-
10th	10-3.0	10-1.6	10-2.0	10-0.3	10-1.6	10-0.6	10-0.3	-
16th	10-3.1	10-2.3	10-1.6	10-0.3	10-2.2	10-0.9	10-0.3	10-0.9

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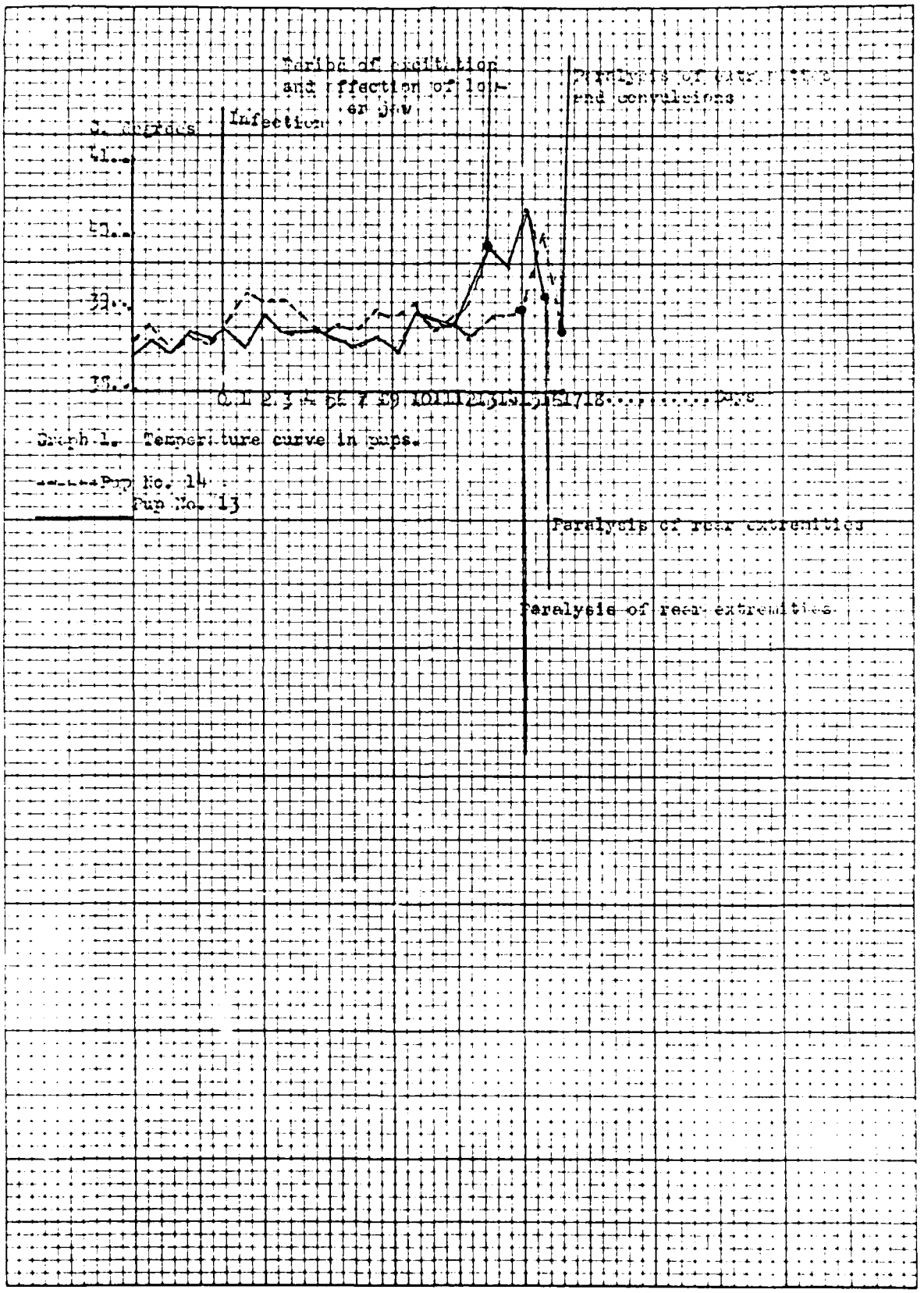
Table 3.. Test of egg strain of rabies virus in tests on pups(dogs)

No. of animal	Strain virus	Dose virus LD50	Dose virus Vol in ml	Place injected	Days incubation	Clinical diagnosis	Titer of virus LD50 in: Brain	Titer of virus LD50 in: ~~Brain~~ Salivary glands	Negri bodies
21	Orig	10-4.2	2.0	Masticatory mus	16	Mixed	10-4.4	10-5.0	ppp
22	"	10-4.2	2.0	"	17	"	10-3.0	10-3.0	ppp
19	Egg 11th pass	10-3.0	0.2	Under hard brain membrane	-	No inf	-	-	-
11	"	10-3.0	0.2	"	10	Mixed	10-3.7	0	ppp
13	"	10-3.0	0.2	"	13	"	10-3.5	0	-
14	"	10-3.0	0.2	"	9	Paralytic form	10-4.2	0	0
15	Egg 14th pass	10-2.3	0.2	"	-	No inf	-	-	-
16	"	10-2.3	0.2	"	7	Mixed	10-4.2	0	0
17	"	10-2.3	0.2	"	-	No Inf	-	-	-
18	"	10-2.3	0.2	"	8	Paralytic	10-5.2	0	0
23	Egg 16th pass	10-3.1	15.0	Under skin	15	No Inf	-	-	-
24	"	10-3.1	10.0	"	12	Paralytic	10-5.0	0	-
25	"	10-3.1	5.0	Thigh muscle	16	"	10-3.5	0	2
26	"	10-3.1	5.0	"	10	"	10-2.2	0	0

Explanation same as Table 1.



Graph 1. Temperature curve in pups.